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HUMAN HAIR STUDIES

Applications of the Microdetermination of Comparative Density*

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Human hair constitutes a type of evidence very frequently encountered by the criminalist, and the question as to whether a given hair or hairs have come from the head of a particular person is a common and important one. The work of Glaister (1), Hausman (2), and others has made relatively simple and quite positive the identification of hair as to species. In the negative sense, human hair may often be definitely shown not to have come from a particular individual. The obvious differences in color, length, and texture serve readily in eliminating a suspected source, and several other more decisive factors have been shown to have a definite eliminative value. The use of hair as a means of positive identification is much more uncertain, and indeed, few experts will venture a definite statement as to the individual origin of a hair.

As a matter of common observation, human head hair is subject to enormous variations between individuals, and to much smaller variations between hairs from the same head. In fact, it is possible to offer a surmise that the hair of every individual is to some detectable extent different from the hair of every other individual, just as is true of virtually every other bodily characteristic which has undergone thorough scrutiny. The quantitative extent of these differences may, however, be very small in a given case, and the means of determining such small differences remain largely unknown. This situation keeps the honest expert from committing himself unreservedly to those instances when there are distinct differences between two samples of hair, establishing that they did not come from the same head. On the positive side, he may say that two samples of hair are similar, or that they are identical to all available tests, and he may evaluate the statistical significance of at least a few of the available tests. He cannot at present

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state definitely that two hairs have come from the same head on the basis of available information. The fact that a hair represents an actual part of the body of an individual from whom it comes, and that it probably carries the individuality of its owner to a definite degree indicates the extreme desirability of making every effort to seek and find every significant factor which may contribute to its more positive individualization. The value of making positive identifications from hair is so obvious as to require no discussion.

Several efforts have been made by Hausman (3), Trotter (4), and others to connect hair with its donors by use of various morphological factors. These have met with very limited success. When it is considered that any differences which exist in significant degree might be expected to affect a variety of morphological, chemical, and physical properties of the hair as was indicated by Kirk (5), it is clear that definitive study of hair has barely been started and that a very large amount of research in this subject can and should be performed. Gamble and Kirk (6) studied and evaluated scale counts and scale count ranges as a means of personal identification from hair. A definite but limited value could be ascribed to these factors. Trotter and Duggins (7) have amplified these results in a recent publication. Greenwell, Willner, and Kirk (8) made a similar extended study of refractive index of hair and established that this physical property also had a definite but limited value in individualization, and was related to sex and race. Aside from studies by Hausman (9) relating scale width to hair diameter in a statistical sense, and the recent work of Trotter (7), there appear to be few or no other studies of the statistical significance of individual characteristics as related to individuality.

In view of the success which attended the application of density comparison of glass (10) and soil (11) in detecting minute differences of source, it appeared worth while to ascertain to what extent the density of hair was a variable factor as between individuals, and as between different hairs of the same head, and whether measurements of this factor could be used in identification of the individual source of a hair. This property, like those previously studied, could be shown to have a very definite but also limited value in determining identities and differences between individual samples of hair.

Experimental

All measurements, both absolute and comparative, of the density of head hairs were made by suspending short segments of the hair in

Figure 1
Spaced Razor Blade Holder for Cutting Segments.

gradient tubes made in a manner similar to that described in earlier publications (10) (11) and modified from the technique of Linderstrøm-Lang (12). The gradient was established by admixing two liquids of different densities in vertical tubes in such a way that the composition of the mixture varied continuously throughout the middle of the tube, each successive increment being lighter than the increment immediately below it. Absolute measurements could be made by comparison with floating beads of known density, and comparative measurements by placing all hair segments to be compared in the same tube, thus giving a direct comparison of the levels at which they floated. Detailed description of the operations is given in appropriate sections below.

Sampling of hair. Most of the hairs studied were taken from a stock collection. Some were obtained directly from individuals available at the time. All but two samples were taken from male Caucasians, and segments of fifty hairs from the same person were placed simultaneously in the gradient in order that statistical treatment of the data would be valid. To establish the validity of sampling, 250 hairs each were taken from five individuals and measured in lots of 50.

Short segments, usually about 1 mm. in length, were cut from each hair. In general, fifty hairs from the same person were aligned and held in place parallel and close to each other by laying them across two parallel strips of scotch tape folded so that half the adhesive surface of

each was exposed. After mounting all hairs, equal segments were cut simultaneously from them by means of a device shown in Figure 1. This consisted of two razor blades held between flat separators and clamped rigidly in place. The two parallel razor blades when pressed on the series of hairs cut a uniform single short segment from each. The length of segment taken could be varied by altering the thickness of the separator between the razor blades. In certain experiments a large number of segments were cut from a single hair. These were prepared by cutting a single segment of the length chosen with a pair of scissors, affixing it under scotch tape and cutting as many more segments as desired with scissors, using the affixed segment as a standard of the proper length.

The cut segments were washed in acetone for 15 minutes to remove surface oil and debris, and dried in the air to prepare them for immersion in the gradient tubes.

Preparation of Gradient Tubes. Several glass tubes 10 mm. in outside diameter and 10 to 14 inches in length were prepared. These were used to contain the gradient liquids. The latter were conveniently made by mixing bromobenzene and nitrobenzene in the ratios shown in column 2 of Table I.

TABLE I.

STOCK SOLUTIONS OF KNOWN DENSITY

Density	Nitrobenzene/Bromobenzene	Ml. per tube
1.310	1.703	4
1.330	1.290	2
1.350	0.987	2
1.370	0.754	2
1.390	0.570	4

To each gradient tube was added the amount of the heaviest solution shown in column 3. To each one was then added the next heaviest liquid in the indicated amount, allowing the liquid to flow gently on the surface of the heavier liquid from a pipet so as to create a minimum of mixing at the interface. This was continued with each solution using a clean pipet for each liquid. Thus in each tube there existed a stepwise variation in density. The tubes were allowed to stand for at least 24 hours to establish by diffusion a uniform density gradient. The tubes so prepared could be used directly for comparative measurements of hair density.

After use, the liquids were partially recovered by filtering, and the density of each was determined with an accurate pycnometer. The recovered liquid could be divided into portions, each of which could

then be readjusted to a desired density by addition of the calculated amount of either bromobenzene or nitrobenzene.

Calibration of Density Gradients. For absolute determinations of density of hair, the most rapid and simple method is a modification of the Linderstrom-Lang procedure for calibration of gradients. That author used small droplets of salt solutions of known density which would distribute themselves at their respective equilibrium positions and provide a scale subject to automatic change with any variation in the gradient. The use of aqueous solutions was not considered advisable in this study for several reasons, and permanent glass standards were prepared as follows.

Soft glass tubing drawn down to capillary size was sealed off in a series of hollow beads containing variable amounts of glass and air. A considerable number of these beads were dropped into a gradient tube made as described for hair studies. Some of the beads rose to the top, some sank to the bottom and some distributed themselves along the gradient. Those in the latter category were selected to represent appropriate divisions of the specific gravity gradient and calibrated by the deJong (13) method as follows. Each bead in turn was introduced into a small tube containing either bromobenzene or nitrobenzene or a mixture of the two, and the density of the entire mixture was varied by addition of the lighter or heavier liquid until on stirring, the bead remained suspended wherever it happened to be when movement of the liquid ceased. At this point the density of the bead and the liquid were the same. Samples of the liquid were withdrawn into a pycnometer, and the density was determined as accurately as possible.

Having calibrated a set of beads, the gradient variation in any tube could be evaluated and the results from different tubes compared with each other by reference to the flexible scale established by the floating beads.

Mounting the Gradient Tube. The gradient tube, held in an upright position by a simple rack, and containing the calibration beads and liquid gradient was then arranged so as to allow convenient reading of the density of suspended hairs. For this purpose a millimeter rule was mounted beside it so that the "zero" of the scale was just opposite the bottom calibration bead. In addition, the placing of a horizontally ruled piece of clear plastic sheet behind the gradient tube and ruler aided greatly in obtaining the reading, and particularly in avoiding parallax due to incorrect eye position, since viewing at an angle through the tube gave a curved appearance to the ruled lines

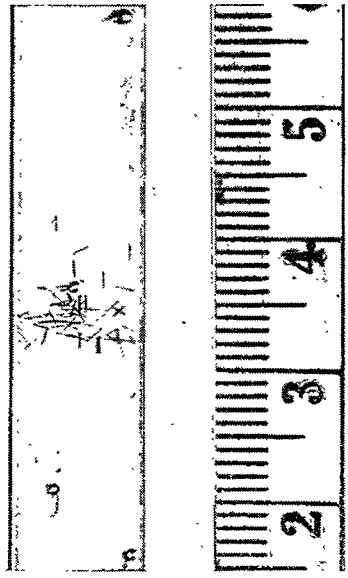

Figure 2
Appearance of Fifty Hair Segments and Calibration Beads.

whereas the lines appeared straight when the eye was at the proper level. The entire assembly was illuminated from the rear by placing behind it a fluorescent light mounted in a reflector, the latter being covered by thin paper or a lightly frosted glass to render the light diffuse and yield a bright uniformly lighted area. This was suitable for photography as well as for visual observation.

By recording the position of each calibration bead with reference to the scale, the density value corresponding to every line of the scale was computed. This was done at the time of reading the position of the hair segments because of the slow alteration of position of the beads as diffusion altered the gradient. Since the beads had a constant density, they continued to mark the location in the tube of a mixture having that density regardless of any change taking place on long standing in the gradient itself. Strong lights or hot objects were found to produce a deleterious effect on the gradient due to the production of convection currents. The fluorescent light used produced no measurable disturbance in the period of time necessary for a set of measurements. It was turned off between times.

Magnification in taking readings was found to be advisable. For reading the position of hair segments a head loupe (Magnifocusser) was sufficient and convenient. It was found desirable to make more detailed observations on individual segments as discussed later. For this

purpose a low power microscope was mounted horizontally in front of the tube. A reading microscope was found very satisfactory, but any such instrument with a long working distance could be used.

Charging the Gradient Tube. The introduction of short hair segments into the gradient tube was an operation requiring special technique. The simplest method found was to use a glass rod which had been drawn down on the end and the tip rounded and slightly flattened in the flame. The top was wet with the gradient liquid, touched to the hair segment which adhered to it, and inserted into the gradient liquid with a plunging action of only a few millimeters, the latter serving to displace the hair from the rod.

After charging the tube with the requisite number of segments, at least 24 hours was allowed to elapse for the hair to come to equilibrium in the tube before readings were taken. This time was chosen as the result of tests of the alterations occurring in time periods up to 60 hours. A sample at equilibrium gave an appearance similar to that shown in Figure 2 in which can be seen four calibration beads and a sample of 50 hair segments.

Results

Early investigations on the application of the density-gradient tube to the study of human hair segments was performed by adding 50 segments of hair from the same individual to a tube to which was also added 1 segment from a different individual. The latter was cut a different length so as to be distinguishable. It was felt that the number of single segments falling in or out of the range of the standard would serve to determine the eliminative value of the method. About 140 individuals were studied, and the results demonstrated that there were factors involved which could not be controlled in so simple a fashion as was first hoped.

It was noted early that distribution of hair from a single individual in the gradient was relatively wide. Microscopic examination of the segments showed clearly that this was due in large part to a difference in the amount of visible medullary material which could be seen in the hair. Without exception those segments which floated high in the gradient tube showed more such medullary material than those further down. Since it is uncommon for an individual to have even approximately regular medullation, throughout his hair, it is clear that in 50 segments cut at random some would contain no such medullary

material, and others would vary in content up to some which would contain it throughout their entire length. It is also a well known fact that individuals vary widely in the degree to which their hair contains such material. This leads to the result that one individual may have little medulla and will give a corresponding close grouping in the gradient tube, while another individual with much medulla will spread rather widely. Because there is no simple method of determining in advance what degree of medullation will be shown by any individual, the only way in which this factor can be easily standardized is to restrict the comparisons to segments which show the presence of no medullary material. This was the method adopted ultimately for comparison of individuals. Non-medullated segments were chosen on the basis of microscopic examination, or a preliminary separation in the gradient tube checked by microscopic observations to assure that no non-medullated segments were discarded because they did not fall within the narrow range of such segments.

So uniform was the effect of medullation in lowering the density of the hair that the possibility is suggested of using this means for studying medullation itself, and its distribution as well as the density. At present, the use of the gradient tube for this type of study has not been investigated sufficiently for any discussion.

The statistical problem presented in this investigation is the determination of the variability of non-medullated segments of a single hair as compared with multiple hairs of the same individual; and the variation of segments from an individual, as compared with segments from another individual, or from the race. Final answers are not completely available to either of these questions, but it is established here with a limited number of male individuals of college age that the variations between individuals is much greater than that between segments from the same individual and that this factor is a useful exclusion method in individualization of human crown hairs. Significant differences may be found between hairs from the same head, partially at least due to the narrower range of a single hair than that of multiple hairs. When medullation is a factor, large differences will be found between single hairs of the same person, and the data in such an instance are not expected to be significant or useful for individualization.

In order to determine the degree of uniformity of hair density of single individuals and to ascertain the reliability of sampling, a detailed study was made of five persons. Five samples of hair were taken from each and each sample consisted of fifty or more segments, each segment

of which was from a different hair. After exclusion of medullated segments, the total number of segments measured was often less than fifty. The data obtained in this study are presented in Table II.

TABLE II.

NON-MEDULLATED HAIRS OF FIVE INDIVIDUALS

Sample No.	No. of Segments	Mean	Theoretical Range ($m \pm 3\sigma m$)
Male, 22 yrs. of age. Hair, light brown, moderate to heavy medullation.			
1	34	1.3338	1.3325-1.3351
2	35	1.3339	1.3326-1.3352
3	48	1.3349	1.3338-1.3360
4	36	1.3341	1.3325-1.3357
5	39	1.3346	1.3331-1.3361
Male, 23 yrs. of age. Hair, very dark brown, slight to medium medullation.			
1	40	1.3362	1.3352-1.3372
2	39	1.3363	1.3349-1.3377
3	47	1.3355	1.3342-1.3368
4	42	1.3363	1.3351-1.3375
5	46	1.3362	1.3355-1.3369
Male, 24 yrs. of age. Hair, black, slight to medium medullation.			
1	41	1.3353	1.3344-1.3362
2	52	1.3351	1.3342-1.3360
3	41	1.3355	1.3347-1.3363
4	49	1.3360	1.3347-1.3373
5	53	1.3358	1.3350-1.3366
Female, 21 yrs. of age. Hair, dark brown, slight to medium medullation, not waved.			
1	40	1.3197	1.3185-1.3209
2	49	1.3200	1.3188-1.3212
3	48	1.3188	1.3175-1,3201
4	53	1.3193	1.3183-1.3203
5	46	1.3197	1.3188-1.3206
Female, 22 yrs. of age. Hair, light blond, slight medullation, not waved.			
1	46	1.3267	1.3252-1.3282
2	43	1.3261	1.3242-1.3280
3	46	1.3261	1.3247-1.3275
4	47	1.3262	1.3246-1.3278
5	48	1.3264	1.3245-1.3283

It will be noted that in every instance, the mean of a subset of determinations fell within a statistical range of means of all sets for that individual. It is clear that with samples of about fifty measurable segments, the reliability of the mean was satisfactory.

The next question to be determined is the significance of differences between individuals when large enough numbers of segments are taken to be representative of each individual. These data must be analyzed statistically, and this was carried out by the following procedure. The densities of the group of segments measured were recorded, and the

mean and standard error calculated for each such group, using for standard error calculations, the well-known formula

$$\sigma M = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

When two means were close to each other, the significance of the difference between them was calculated from the formula

$$\sigma D = \sqrt{\sigma M_1{}^2 - \sigma M_2{}^2}$$

where σD is the standard error of the difference, σM_1 and σM_2 are the standard errors of the means being compared. If the ratio of the difference between means to the standard error of the difference, i.e. $D/\sigma D$ was greater than 3, it was concluded that the difference was significant. When the ratio was equal to 3, it was assumed that the difference was significant in all but about three cases out of 1000 which is still a high probability of significance. Below a ratio of three, it was assumed that the difference was not significant and that it could not be stated that two sets of data represented different "populations." Many authorities accept a ratio of 2 as indicating significance because the probability of the significance is still of the order of 95 percent. The data taken from 14 people to study variations between them is summarized in Table III. The number of segments measured is given there

TABLE III.

COMPARISON OF MULTISEGMENT DENSITIES OF NON-MEDULLATED HAIR OF VARIOUS INDIVIDUALS

Individual	Mean Density	Standard Error σM	Statistical Range $m \pm 3\sigma M$	Indistinguishable Samples
1	1.3426	0.000418	1.3413-1.3439	3, 2, 5
2	1.3427	0.000224	1.3420-1,3434	1, 5
3	1.3415	0.000266	1.3407-1.3423	6, 1
4	1.3449	0.000270	1.3436-1.3452	11
5	1.3430	0.000358	1,3419-1.3441	1, 2
6	1.3410	0.000303	1.3401-1.3419	10, 3
7	1.3356	0.000231	1.3349-1.3363	8
8	1.3354	0.000120	1.3350-1.3358	7
9	1.3475	0.000114	1.3472-1.3478	12
10	1.3399	0.000254	1.3397-1.3413	6
11	1.3443	0.000220	1.3436-1.3450	4
12	1.3472	0.000123	1.3468-1.3476	13, 9
13	1.3467	0.000182	1.3461-1.3473	12
14	1.34885	0.000077	1.3486-1.3491	—

along with the means and the standard errors of the various means. All of the individuals listed in this table were male, Caucasian college students. It will be noted from the data that the number of individuals from which a given hair sample could not be distinguished was 1.4 on

the average which, in a total number of 14 samples, is 10 percent. Thus, it can be stated that on the basis of the data obtained from these particular 14 similar individuals, one person on the average could be distinguished from 10 possible individuals. Revision of this value with the study of larger numbers of individuals is probable, but a change in order of magnitude is very unlikely. It should be noted that this study makes no contribution to the probable effect of age, race, or sex, with the exception of the two sets of data in Table II which are not shown in Table III, and in which female hair appears to range somewhat lower than male hair.

Since a single hair may be found in evidence, it is important to establish whether it is representative of an entire head of hair. This would seem to be doubtful in view of other factors which have not been found to be as reproducible as this. Certainly, when the hair is heavily medullated, it is unlikely to agree with other hairs with different degrees of medullation. If not medullated, it should agree more closely with other non-medullated hairs or with their average. A limited number of single hairs were measured in order to evaluate this point. Without listing the data, it suffices to state that the means of most of the single hairs did agree with the mean of multiple hairs within the statistical limits defined above. There were a few that were somewhat outside of the statistical range. Because of the small number of single hairs which were studied, a final conclusion seems unwarranted without further study. More investigation of other factors that affect hair density, and a broader study of individuals is planned.

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